

## REMARKS

### Interview

Applicants thank the Assistant Examiner and the Primary Examiner for the courtesy extended during the personal interview held August 13, 2002.

### Supplemental IDS

Further to the Information Disclosure Statement filed March 7, 2002, applicants file concurrently herewith a Supplemental Information Disclosure Statement that makes of record references recently cited in a communication from a foreign patent office in a counterpart international application.

### Status of and amendment to the claims

Claims 25 - 77 are pending and have been rejected.

Claim 39 is cancelled herein without prejudice.

Claims 25, 26, 32, 50, 51, 52, 57, 58, 72 and 75 are amended herein for the reasons, unrelated to patentability, further described below. Support for the claim amendments may be found throughout the specification, and particularly as follows; no new matter has been added.

Claim 25 has been amended to recite explicitly that the cellular proteins present in admixture with the targeted nucleic acid and sequence-altering oligonucleotide are "cellular repair proteins". This clarification effects no alteration in scope, the

specification making clear throughout that the sequence-altering oligonucleotides of the present invention interact with the nucleic acid repair machinery of the cell. See, e.g., p. 6, line 31; p. 9, lines 30 - 31.

Claim 25 has further been amended additionally to recite that the cellular repair proteins are "present within selectively enriched cells, cells in culture, or cell-free extracts".

Targeted alteration of selectively enriched cells is particularly described in Example 3, pages 37 - 38 (alteration of CD34<sup>+</sup> hematopoietic stem cells selectively enriched from umbilical cord blood). Targeted alteration of cells in culture is described throughout the specification, and particularly at p. 12, lines 20 - 21; p. 13, lines 14 - 16; p. 21, lines 15 - 24; p. 22, lines 13 - 17; and Table 9. Targeted alteration in cell-free extracts is described particularly at p. 18, line 11 - p. 19, line 20; p. 22, line 20; p. 4, lines 29 - 30; p. 7, line 6; and elsewhere throughout the specification.

Claim 52 is amended to include "rodents" among its listed non-human mammals. Support may be found particularly at p. 6, line 18.

Claim 72 is amended particularly to clarify that the stem cell targeted for alteration of a human beta globin gene is a "human hematopoietic stem cell"; support may be found particularly in Example 6, particularly p. 64, line 20.

Written Description Rejections Under 35 U.S.C.  
§ 112, First Paragraph Are in Error and Should  
be Withdrawn

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The Examiner rejects claims 25 - 77 under 35 U.S.C. § 112, ¶ 1 on grounds that the specification "does not disclose oligonucleotides having an internally unduplexed domain of at least 8 contiguous deoxyribonucleotides"; in particular, the Examiner notes that "it is not apparent in the figures that any of the oligonucleotides comprise an internally unduplexed domain of at least 8 contiguous deoxyribonucleotides." Applicants respectfully disagree.

In "[t]he original RNA-DNA double hairpin chimera design, e.g., as disclosed in U.S. Patent 5,565,350, . . . [t]he double-stranded targeting region is made up of a 5 base pair DNA/DNA segment bracketed by 10 base pair RNA/DNA segments."<sup>1</sup> Representative chimeras are shown in FIGS. 1A (structure I), 1B (structure II), 1C (chimeric oligos "KanGG" and "TetΔ208T"), 1D ("KanGG chimera"), and 8 ("HygGG/Rev"). Although the representational approach varies as among these figures, in each case the internal duplexing of the chimera's repair region is clearly shown.

The sequence-altering oligonucleotides of applicants' invention are very different.

We have discovered that single-stranded oligonucleotides having a DNA domain surrounding the targeted base, with the domain preferably central to the poly- or oligo-nucleotide . . . are able to alter a target genetic sequence and with an efficiency that is higher than chimeric

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<sup>1</sup> Specification p. 23, lines 7 - 10 (emphasis added).

RNA-DNA double hairpin oligonucleotides disclosed in US Patent 5,565,350.<sup>2</sup>

The DNA domain is preferably fully complementary to one strand of the gene target, except for the mismatch base or bases responsible for the gene alteration or conversion events. On either side of the preferably central DNA domain, the contiguous bases may be either RNA bases or, preferably, are primarily DNA bases. The central DNA domain is generally at least 8 nucleotides in length. The base(s) targeted for alteration in the most preferred embodiments are at least about 8, 9, 10 bases from one end of the oligonucleotide.<sup>3</sup>

In explicit contrast to the internally duplexed chimeras,

[t]he[] oligonucleotides . . . [of the present invention] are . . . not designed to form a stable internal duplex structure within the oligonucleotide.<sup>4</sup>

This is clearly illustrated in FIGS. 1A and 1B: in contrast to chimeric structures I and II, which are explicitly duplexed in their respective targeting regions (the regions having a mismatched base, "▲"), single-stranded structures III, V, VI, VII, VIII, IX, X, XI, XII and XIII are explicitly and correctly drawn to show the absence of such internal duplex.

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<sup>2</sup> Specification, p. 7, lines 7 - 11 (emphasis added).

<sup>3</sup> Specification p. 7, lines 17 - 22 (emphasis added).

<sup>4</sup> Specification p. 7, lines 27 - 28.

Applicants respectfully submit that the specification provides ample written description support for the questioned phrase, that the rejection is in error, and that the rejection should be withdrawn.

Rejections Under 35 U.S.C. § 112, Second  
Paragraph Are in Error, Have Been Obviated, and  
Should be Withdrawn

The Examiner rejects claims 25 - 77 under 35 U.S.C. § 112, ¶ 2 on a number of separate grounds. Applicants respectfully traverse each of the rejections in the separate sections below.

"in the presence of cellular proteins"

In a first rejection under 35 U.S.C. § 112, second paragraph, the examiner rejects claim 25 on the ground that the phrase "in the presence of cellular proteins" renders the claims indefinite "because it is unclear if the cellular proteins are in a cell, or in cell extracts."

Applicants respectfully traverse the rejection.

"The test for definiteness is whether one skilled in the art would understand the bounds of the claim when read in light of the specification. If the claim read in light of the specification reasonably apprise[s] those skilled in the art of the scope of the invention, section 112 demands no more." *Miles Labs., Inc. v. Shandon Inc.*, 997 F.2d 870, 875 (Fed. Cir. 1993) (internal citations omitted). It matters not that the term is broad, so long as the metes and bounds of territory compassed by

the claim are clear. "[B]readth is not to be equated with indefiniteness. . . ." *In re Miller*, 441 F.2d 689, 393 (CCPA 1971), *quoted with approval in Union Pacific Resources Co. v. Chesapeake Energy Corp.*, 236 F.3d 684, 692 (Fed. Cir. 2001); *Ex Parte Blalock*, 2002 WL 465335 (Bd. Pat. App. & Interf. 2002).

As the Examiner notes, the phrase "in the presence of cellular proteins" comprehends both proteins within the cell and cellular extracts. Applicants agree: the phrase is generic to each of the alternatives identified by the Examiner. That the phrase is broad does not, however, render the claim indefinite. Just as the Examiner has been able to articulate the alternatives that fall within the scope of the claim, so too the skilled artisan will readily understand the metes and bounds of the claim. The rejection is in error and should be withdrawn.

Solely to expedite prosecution, however, applicants amend the claims herein explicitly to recite that the oligonucleotide is combined with the targeted nucleic acid "in the presence of cellular repair proteins present within selectively enriched cells, cells in culture, or cell-free extracts", and respectfully submit that the rejection has additionally been obviated and should be withdrawn.

"an internally unduplexed domain"

In a second rejection for indefiniteness, the Examiner rejects claim 25 for recitation of "an internally unduplexed domain", arguing that "it is unclear if the unduplexed domain is not duplexed with any part of the oligonucleotide, or if the domain does not form a duplex structure with the target nucleic acid."

The specification states that the oligonucleotides of the present invention "are . . . single-stranded and are not designed to form a stable internal duplex structure within the oligonucleotide." Specification p. 7, lines 25 - 26 (emphasis added). This statement makes abundantly clear that the Examiner's first construction is correct.<sup>5</sup>

Applicants respectfully submit that those skilled in the art would understand the bounds of the claim when read in light of the specification, that the phrase thus satisfies the requirements of § 112, ¶ 2, and that the rejection is in error and should be withdrawn.

"said oligonucleotide DNA domain"

The Examiner further rejects claim 25 for reciting "said oligonucleotide DNA domain" in line 11, arguing that "there is no prior reference in the claim to any 'oligonucleotide DNA domain.'"

Applicants respectfully disagree.

Lines 5 - 7 of claim 25 recite that "said oligonucleotide ha[s] an internally unduplexed domain of at least 8 contiguous deoxyribonucleotides". A "domain of at least 8 contiguous deoxyribonucleotides" is a "DNA domain", and lines 5 - 7 of the claim thus provide the required antecedent basis for later recitation in the claim of "said oligonucleotide DNA domain".

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<sup>5</sup> In addition, the specification consistently and repeatedly distinguishes oligonucleotides used in applicants' methods from molecules that are "folded by complementarity into a double hairpin conformation", specification p. 2, lines 12 - 15 (emphasis added). See also specification p. 6, lines 16 - 17; specification p. 7, lines 3 - 6. These portions of the specification further clarify that "the unduplexed domain is not duplexed with any part of the oligonucleotide", and that the Examiner's first construction is correct.

Solely to expedite prosecution, however, applicants herein amend claim 25 to replace "oligonucleotide DNA domain" with "internally unduplexed deoxyribonucleotide domain", which adheres more closely to the exact language of the initial reference. There is no change in claim scope.

Claims 57, 58, and 75 are rejected on identical grounds; for identical reasons and to identical effect applicants herein amend each of these claims to recite "internally unduplexed deoxyribonucleotide domain" in lieu of "DNA domain".

The Examiner also rejects claims 60 - 62, 64 - 66, and 69 for recitation in lines 1 - 2 of "oligonucleotide DNA domain". This appears to be a clerical oversight: in applicants' version of the amendment filed March 7, 2002 first adding these claims, the phrase does not appear in any of claims 60 - 62, 64 - 66, or 69.

For the aforesaid reasons, applicants respectfully submit that rejections of claims 25, 57, 58, 75, 60 - 62, 64 - 66, and 69 under 35 U.S.C. § 112, ¶ 2 are in error, have been obviated, and should be withdrawn.

"chromosome"

The Examiner rejects claim 33 under 35 U.S.C. § 112, second paragraph, arguing that there is insufficient antecedent basis for recitation of the word "chromosome".

Claim 33 depends from claim 32, which recites "wherein said genomic DNA is chromosomal". Applicants submit that, notwithstanding the change in word form, recitation of "chromosomal" provides adequate antecedent basis for later recital of "chromosome". Solely to expedite prosecution, however,



applicants amend claim 32 to recite "wherein said genomic DNA is in a chromosome", thus providing *ipsis verbis* antecedent basis for claim 33's later recitation of "chromosome". The amendment effects no change in the scope of claim 32 or claim 33.

Rejection is in error, has been obviated, and should be withdrawn.

"mammal"

The Examiner rejects claim 52, arguing that there is no antecedent basis for the claim's recitation of "mammal" in line 1.

Claim 52 depends from claim 51. Claim 51's recitation of "wherein said eukaryotic cell is a mammalian cell" provides sufficient antecedent basis for claim 52's recital of "mammal", notwithstanding the difference in word forms. In order solely to expedite prosecution, however, applicants herein amend claim 51 to recite "wherein said eukaryotic cell is from a mammal", providing *ipsis verbis* antecedent basis for claim 52's recitation of "mammal". The amendment effects no change in scope in claim 51 or claim 52.

The rejection is in error, has been obviated, and should be withdrawn.

**Rejection Under 35 U.S.C. § 102 Is In Error And  
Should Be Withdrawn**

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The Examiner rejects claims 25 - 77 under 35 U.S.C. § 102(b) as anticipated by Sayers et al., *Nucl. Acids Res.*

16(3):791-802 (1988) ("Sayers"). The rejection is in error and should be withdrawn.

"To anticipate, every element and limitation of the claimed invention must be found in a single prior art reference, arranged as in the claim." *Karsten Mfg. Corp. v. Cleveland Golf Co.*, 58 USPQ2d 1286, 1291 (Fed. Cir. 2001); *Scripps Clinic & Research Foundation v. Genentech, Inc.*, 927 F.2d 1565, 1576, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991).

As examined, the method of applicants' claim 25 requires that a single-stranded oligonucleotide be combined with targeted nucleic acid in the presence of cellular proteins. The single-stranded oligonucleotide has at least one terminal modification selected from the group consisting of: (i) at least one terminal locked nucleic acid (LNA), (ii) at least one terminal 2'-O-Me base analog, and (iii) at least three terminal phosphorothioate linkages.

Sayers anneals an oligonucleotide to a single stranded M13 template in the presence of a purified polymerase and extends the primer in the presence of "each dNTP with one dNTP [dCTP] replaced by its corresponding phosphorothioate analogue"<sup>6</sup>. Although incorporation of the one thiolated dNTP analogue creates periodic phosphorothioate linkages in the extension product, the oligonucleotide as combined with target is itself modified only by attachment of a 5' phosphate; it lacks the modifications recited in applicants' claims.

Lacking at least one element of applicants' claim 25, Sayers cannot anticipate the claim. The reference also cannot

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<sup>6</sup> Sayers, p. 794, lines 3 - 5.

anticipate claims 26 - 74, which depend directly or indirectly from claim 25 and thus incorporate all limitations of the independent claim, 37 C.F.R. § 1.75(c). The rejection is in error and should be withdrawn.

Solely to expedite prosecution, and without change in claim scope, applicants amend claim 25 herein explicitly to recite that the oligonucleotide is combined with the targeted nucleic acid "in the presence of cellular repair proteins", an element clearly absent from Sayers. Sayers does not and cannot anticipate claims 25 - 74, and the rejection should be withdrawn.

In the method of applicants' independent claim 75, the sequence-altering oligonucleotide "includes the sequence of any one of SEQ ID NOS: 1 - 4340." Sayers' oligonucleotide does not include any of the referenced sequences. Lacking such element, Sayers cannot anticipate claim 75 or claims 76 - 77 which depend therefrom; the rejection is in error and should be withdrawn.

Solely to expedite prosecution, and without change in claim scope, applicants amend claim 75 herein explicitly to recite that the oligonucleotide is combined with the targeted nucleic acid "in the presence of cellular repair proteins", an element clearly absent from Sayers. Sayers does not and cannot anticipate claims 75 - 77, and the rejection should be withdrawn.

Rejections Under 35 U.S.C. § 112, ¶ 1 For  
Inadequate Scope of Enablement Are In Error  
And Should Be Withdrawn

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The Examiner rejects claims 25 - 77, all of the claims presently pending, on grounds that applicants' specification

provides inadequate scope of enablement.<sup>7</sup> The Examiner bases this conclusion in part upon the proposition that

[t]he specification has only one working example of using oligonucleotides for successful targeted alteration. The example disclosed is of successful alteration of an episomal nucleic acid in yeast (Example 2, p. 32).<sup>8</sup>

Applicants respectfully disagree.

Tables 1, 2 and 3 (pages 26, 28, and 29, respectively) present results demonstrating targeted gene alteration of an episomal target in the presence of cell-free extracts from a variety of mammalian cell lines, including human HUH7 liver cells and murine embryonic fibroblast (MEF) lines. Table 4 (page 30) presents results demonstrating targeted gene alteration of an episomal target in the presence of cell-free extracts from a variety of plant genera. Table V (page 31) presents results demonstrating successful gene alteration in cell-free extracts prepared from yeast (*Saccharomyces cerevisiae*).

Example 2 demonstrates successful targeting of an episomal target in yeast cells (see Table 6, page 36). FIG. 5A is a confocal micrograph that demonstrates successful targeting of an episomal target in HeLa cells.

Applicants respectfully submit that the specification enables those skilled in the art to make and use applicants' invention, without undue experimentation, across the entirety of

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<sup>7</sup> Although the rejection is not expressly so stated, the Examiner's recognition that applicants' working example demonstrates "successful targeted alteration", Office Action at 7, makes clear that the rejection is predicated on inadequate scope, rather than on a lack, of enablement.

<sup>8</sup> Office Action, p. 7 (emphasis omitted).

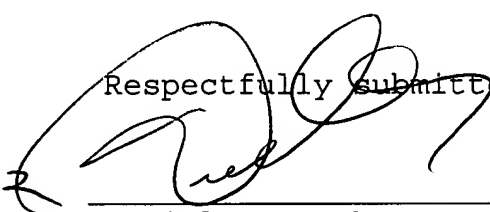
the claims' scope, and that the rejection is in error and should be withdrawn.

CONCLUSION

Applicants respectfully submit that the claims are in good and proper form for allowance, and earnestly solicit the same. If the Examiner believes, however, that any matters remain outstanding, applicants invite the Examiner to call the undersigned attorney of record.

Respectfully submitted,

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Amended Claims

Marked-Up Pursuant to 37 C.F.R. § 1.121(c)(1)(ii)

25 (once amended). A method of targeted sequence alteration of a nucleic acid, comprising:

combining the targeted nucleic acid, in the presence of cellular repair proteins present within selectively enriched cells, cells in culture, or cell-free extracts, with a single-stranded oligonucleotide 17 - 121 nucleotides in length, said oligonucleotide having an internally unduplexed domain of at least 8 contiguous deoxyribonucleotides,

wherein said oligonucleotide is fully complementary in sequence to the sequence of a first strand of the nucleic acid target, but for one or more mismatches as between the sequences of said [oligonucleotide DNA] internally unduplexed deoxyribonucleotide domain and its complement on said target nucleic acid first strand, each of said mismatches positioned at least 8 nucleotides from said oligonucleotide's 5' and 3' termini, and

wherein said oligonucleotide has at least one terminal modification selected from the group consisting of: at least one terminal locked nucleic acid (LNA), at least one terminal 2'-O-Me base analog, and at least three terminal phosphorothioate linkages.

26 (once amended). The method of claim 25, wherein said sequence alteration is a substitution of at least one base.

32 (once amended). The method of claim 31, wherein said genomic DNA is [chromosomal] in a chromosome.

50 (once amended). The method of claim 49, wherein said human cell is selected from the group consisting of liver cell, lung cell, colon cell, cervical cell, kidney cell, epithelial cell, cancer cell, and stem cell[, embryonic stem cell].

51 (once amended). The method of claim 45, wherein said eukaryotic cell is [a mammalian cell] from a mammal.

52 (once amended). The method of claim 51, wherein said mammal is selected from the group consisting of: rodent, mouse, hamster, rat, and monkey.

57 (once amended). The method of claim 25, wherein the sequences of said [oligonucleotide DNA] internally unduplexed deoxyribonucleotide domain and of the target nucleic acid first strand are mismatched at a single nucleotide.

58 (once amended). The method of claim 25, wherein the sequences of said [oligonucleotide DNA] internally unduplexed deoxyribonucleotide domain and of its complement on the target nucleic acid first strand are mismatched at two or more nucleotides.

72 (once amended). The method of claim 71, wherein said human beta-globin gene is targeted in a human hematopoietic stem cell.

75 (once amended). A method of targeted sequence alteration of a nucleic acid, comprising:

combining the targeted nucleic acid, in the presence of cellular repair proteins present within selectively enriched cells, cells in culture, or cell-free extracts, with a single-stranded oligonucleotide 17 - 121 nucleotides in length, said oligonucleotide having an internally unduplexed domain of at least 8 contiguous deoxyribonucleotides,

wherein said oligonucleotide is fully complementary in sequence to the sequence of a first strand of the nucleic acid target, but for one or more mismatches as between the sequences of said [oligonucleotide DNA] internally unduplexed deoxyribonucleotide domain and its complement on said target nucleic acid first strand, each of said mismatches positioned at least 8 nucleotides from said oligonucleotide's 5' and 3' termini, and

wherein said oligonucleotide has at least one terminal modification and includes the sequence of any one of SEQ ID NOs: 1 - 4340.